The cdk/pRB/E2F pathway in human cancer

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Mutations in the retinoblastoma gene (RB-1) have been described in a wide variety of neoplasms. Furthermore, many cancers that retain a wild-type RB-1 allele harbor mutations that lead to the untimely phosphorylation and hence inactivation of the RB-1 gene product pRB. Examples of such mutations include amplification of cyclin D1, gain of function cdk4 missense mutations, and a homozygous deletion of the p16 cdk inhibitor. In summary, inactivation of pRB may be a *sine qua non* of cancer.

How different cyclin-dependent kinases (CDKs) recognize substrates in general, and pRB, is largely unknown. We have identified a short, collinear peptide sequence that serves as a cyclin/CDK2 substrate-recognition motif. This sequence, as a synthetic peptide, will specifically block the phosphorylation of pRB by cyclin/CDK2 complexes. This peptide can be viewed as a lead compound for the development of a small molecule CDK2 inhibitor. We are currently asking whether this paradigm can be extended to other CDKs such as CDK4.

We and others have linked the ability of pRB to regulate cell cycle progression to its ability to bind to members of the E2F transcription factor family. pRB/E2F complexes actively repress the transcription of E2F-responsive genes. Thus, E2F responsive genes should be more actively transcribed in tumor cells than in normal cells because of the loss of pRB/E2F transcriptional repressor complexes and liberation of free, transcriptionally active E2F. To test this directly, we, in collaboration with Dr. Howard Fine, constructed an adenoviral vector in which a reporter gene was placed under E2F control. As expected, the infection of tumor cells *in vivo* with this virus led to high levels of reporter activity. In contrast, reporter activity was not measurable in surrounding normal cells despite evidence of viral infection. Thus, deregulation of E2F can be measured in tumors *in vivo* and may be exploited to selectively drive gene expression in tumor cells.

pRB can both regulate the cell cycle and promote differentiation. We recently created pRB mutants that cannot bind to E2F, cannot repress transcription, and yet retain the ability to promote differentiation in model systems. Interestingly, at least two naturally occurring, partially penetrant pRB also have this phenotype. In the simplest view, the lower risk of retinoblastoma associated with these mutants reflects the fact that they retain a pRB tumor suppressor activity, namely, the ability to promote differentiation. We are currently studying the biochemical mechanisms underlying this pRB activity.

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